

LITERATURE REVIEW ON EFFICACY OF DISINFECTION METHODS BY SPECIES

The following appendix outlines the effectiveness of various disinfection methods on specific species, and includes citations for determinations. It is a working document that will be updated as new findings are made. If you have any new citations to add, please send suggestions to Maureen Ferry at maureen.ferry@wisconsin.gov.

Key:

☑= Effective- Eliminates spp when applied at rates outlined in the manual code.

⊗=Not Effective- Requiring higher rates and/or longer time periods than outlined in code to eliminate spp.

Ⓡ= Research Needed- No/insufficient sources or references found.

?= Lit Review Needed- Existence of sources or references not yet known.

Supporting references are enumerated in superscript. Symbols shown without references depict commonly shared knowledge wherein references or studies to validate may exist but have not yet been found.

Table 1 Efficacy of treatment methods for macrophytes and algae.

AIS	Steam Cleaning (212°F)	Hot Water (140°F)	Drying (5 days)	Chlorine (500 ppm, 10 min)	Virkon (2:100 solution, 20 min)	Freezing (26°F [†])
Curly Leaf Pondweed	Ⓡ	Ⓡ	☑ ^{3,55}	Ⓡ	Ⓡ	⊗ ⁵²
Curly Leaf Pondweed Turion	☑	☑ ⁵³	⊗ ³	Ⓡ	Ⓡ	Ⓡ
Eurasian Watermilfoil	☑	☑ ¹⁵	☑ ^{12,55}	Ⓡ ^{57*}	Ⓡ	⊗ ^{58*}
Eurasian Watermilfoil Seed	Ⓡ	Ⓡ	⊗ ⁵⁶	Ⓡ	Ⓡ	Ⓡ
Hydrilla	Ⓡ	Ⓡ	☑ ^{55*,59,60*, 61}	Ⓡ	Ⓡ	Ⓡ
Yellow Floating Heart	Ⓡ	Ⓡ	⊗ ^{62*}	Ⓡ	Ⓡ	Ⓡ
Starry Stonewort	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ
Didymo	☑	☑ ^{13,48}	☑ ^{13,48}	☑ ^{13,48,49,50, 51}	☑ ¹	☑ ⁴⁸

*Additional details:

⁵⁵ Hydrilla reported as “fasting drying plant” of 10 species tested; however, additional viability testing not done due to state transport laws

⁵⁷ Study looked at substantially lower concentrations.

⁵⁸ EWM seeds likely experience increased viability after freezing

⁶⁰ Study only tested twigs for up to 24hrs

⁶² *N. peltata* seeds show high tolerance to desiccation

Table 2 Efficacy of treatment methods for invertebrates.

AIS	Steam Cleaning (212°F)	Hot Water (140°F)	Drying (5 days)	Chlorine (500 ppm, 10 min)	Virkon (2:100 solution, 20 min)	Freezing (26°F [†])
Faucet Snail	✓	✓ ^{18*}	⊗ ^{18,35}	⊗ ¹⁸	Ⓡ ¹⁸	✓
New Zealand mud snail	✓	✓ ^{4,65*}	✓ ^{6*,66*}	⊗ ^{21, 77*}	✓ ^{10*, 76, 77, 78}	✓ ^{4,6*, 77}
Quagga Mussel (Adults)	✓ [†]	✓ ^{7*,16*}	✓ ^{14*,67}	✓	✓ ⁹	✓
Quagga Mussel (Veligers)	✓ [†]	✓ ^{4,17}	✓ ^{69*, 78*}	✓	✓ ⁹	✓
Zebra Mussel (Adult)	✓ [†]	✓ ^{7*,8*,54,67}	✓ ^{14*,25*,67}	✓ ^{11,19,22}	Ⓡ	✓ ^{25,27,67,68}
Zebra Mussel (Veligers)	✓ [†]	✓ ⁴	Ⓡ	✓	Ⓡ	✓
Asian Clam	✓	✓ ^{4,37,41,42,43}	⊗ ^{4,44*,45}	⊗ ^{36*,37*,38*,39*,40}	✓ ²³	✓ ^{46*}
Spiny Water Flea (Adult)	✓	✓ ^{7*,47*}	✓ ⁴	✓ ⁷⁷	✓ ⁷⁷	✓ ⁷⁷
Spiny Water Flea (Resting Eggs)	✓	✓ ^{2*}	✓ ^{2*}	⊗ ^{2, 77*}	✓ ⁷⁷	✓ ^{2*}
Bloody Red Shrimp	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ
Rusty Crayfish	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ

*Additional details:

² Frozen in water, not just in air; Hot water: 50°C (122°F) for >5 min (or 1 min at >50°C); Drying: ≥ 6 hr @ 17°C (63°F)

⁶ Drying: Must ensure hot and dry environment (>84°F for 24hrs; ≥ 104°F (40°C) for >2 hours); Freezing: ≤ 27°F (-3°C) for 1 to 2 hours

⁷ >43°C (110°F) for 5-10 min

⁸ ≥ 140°F (60°C) for 13 to 10 seconds

¹⁰ 2% solution (77 grams/1 gal water) for 15-20 min

¹⁴ Adult *Dreissena* may survive overland transport for 3-5 days

¹⁶ $\geq 140^{\circ}\text{F}$ (60°C) for 5 to 10 seconds

¹⁸ 50°C (122°F) for ≥ 1 min

²⁵ Must ensure hot and dry environment ($>25^{\circ}\text{C}$ for at least 2 days, or 5 days when humidity is high)

³⁶ Long exposure times (2-28 days) at low rates (0.2-40 mg/L)

³⁷ Short exposure time (30 min) at low rates (0, 5, 7.5, & 10 mg/L)

^{37,41-43} Morality at $35-43^{\circ}\text{C}$ ($95-110^{\circ}\text{F}$)

³⁸ Long exposure time (14-28 days) to low rates (0.25-0.4 mg/L)

³⁹ Long exposure time (28-32 days) to low rates (0.2-1 mg/L)

⁴⁴ 2 weeks need for mortality

⁴⁶ Lethal temperature reported at 0°C ; freezing is a possible control method which warrants research

⁴⁷ $>38^{\circ}\text{C}$ (100°F) for 12 hrs

⁶⁵ $>50^{\circ}\text{C}$ (122°F) for 15 seconds

⁶⁶ Dry in full sunlight for ≥ 50 hrs

⁶⁹ Veligers experienced 100% mortality after 5 days under summer temperature conditions, and after approximately 27 days under autumn temperature conditions

[†] Mentioned as effective in DiVittorio et al 2010, however no reference or study provided to validate claim

Table 3 Efficacy of treatment methods for viruses and diseases.

AIS	Steam Cleaning (212°F)	Hot Water (140°F)	Drying (5 days)	Chlorine (500 ppm, 10 min)	Virkon (2:100 solution, 20 min)	Freezing ($26^{\circ}\text{F}^{\dagger}$)
Spring Viremia of Carp virus (SVCv)	✓	✓ ^{29*,30,31*,64}	⊗ ^{4*}	✓ ^{28*,29*,30,64}	✓ ^{28*}	⊗ ²⁹
Largemouth Bass virus (LMBv)	®	®	®	✓ ^{24*,28*}	✓ ^{24,28*}	⊗ ³²
Viral Hemorrhagic Septicemia virus (VHSv)	✓	✓ ^{4,72,74*}	✓ ^{4,72,74*}	✓ ^{28*}	✓ ^{28*,72}	✓ ^{26,29,63*} ⊗ ⁷⁴
Lymphosarcoma	®	®	®	✓	®	®
Whirling Disease	✓ ^{33*}	⊗ ^{20*,33*,72}	✓ ^{5,33*}	✓ ^{5*,20*,28*,33*}	®	✓ ^{5*,33*}
Heterosporis	®	®	✓ ^{34*}	✓ ^{34*}	®	✓ ^{34*}

*Additional details:

⁴ Drying of >28 days at 70°F needed

⁵ Bleach 500 mg/L for >15 min; Freezing at either -20°C or -80°C for 7 days or 2 months

²⁰ Heat @ 90°C for 10 min; Bleach at 1600 ppm for 24hrs, or 5000 ppm for 10 min

²⁴ 10% bleach/water solution

- ²⁸ For SVC: Bleach = 500mg/L for 10 min; Virkon = 0.5-1% for 10 min, or 0.1% for 30 min
 For VHS: Bleach = 200-500mg/L for 5 min; Virkon=0.5-1% for 10 min
 For Whirling Disease: Bleach = 500 mg/L for 10-15 min; Virkon = 0.5-1% for 5 min
 For Ranavirus (LMBv): Bleach = 500 mg/L for 15 min; Virkon = 0.5-1% for 1 min
- ²⁹ Hot water = 56°C for 30 min; Bleach = 520 mg/L for 20 min
- ³¹ Hot water 60°C (140°F) for 30 min = 99.9% mortality
- ³³ Freeze = 105 min @ -20°C; Desiccation = 60 min @ 19-21°C; Hot water (submerged in test tubes) = 5 min @ 75°C;
 Bleach = 13ppm for >10 min, 131ppm for >1 min
- ³⁴ Freeze 24 hrs @ -4°F; Bleach=3cups/5 gal of water; Dry = > 24hrs
- ⁶³ Will not completely kill virus but will reduce infectivity or virus titres by >90%
- ⁷³ 122°F (50°C) for 10 minutes, or 122°F (50°C) for 10 minutes
- ⁷⁴ study done on IHNH virus (similar to VHSV); dry gear for 4 days at 21°C (70°F)

References

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2. Branstrator, D. K., L. J. Shannon, M. E. Brown, and M. T. Kitson. 2013. Effects of chemical and physical conditions on hatching success of *Bythotrephes longimanus* resting eggs. *Limnology and Oceanography* 58(6):2171-2184.
Frozen in water, not just in air; Hot water: 50°C (122°F) for >5 min (or 1 min at >50°C); Drying: ≥ 6 hr @ 17°C (63°F). Chlorine solutions of 3400 mg L⁻¹ had no impact on hatching success when exposed for up to 5min.
3. Bruckerhoff, L., J. Havel, and S. Knight. 2013. *Survival of Invasive Aquatic Plants After Air Exposure and Implication for Dispersal by Recreation Boats*. Unpublished data.
Studied the impacts of drying on the viability of Eurasian watermilfoil and curly-leaf pondweeds. For Eurasian watermilfoil, single stems were viable for up to 24hrs while coiled strands were viable for up to 72hrs. For curly leaf pondweed, single stems were viable for 18hrs, and turions were still viable after 28 days of drying.
4. USFS Intermountain Region Technical Guidance. 2014. Preventing Spread of Aquatic Invasive Organisms Common to the Intermountain Region.
http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5373422.pdf
Outlines guidance to avoid spread of AIS during fire management and suppression activities. Recommends treatments for various species based on a literature review; references are outlined in this guidance. For quagga and zebra mussel adults and larvae: ≥140°F (60°C) hot water spray for 5 to 10 seconds, or hot water immersion of ≥120°F (50°C) for 1 minute. Freeze at 0°C for adults. Dry for 5 days. 0.5% bleach solution rinse. 2% Virkon Aquatic solution for 10 minutes.

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This publication provides an overview of major concepts in biosecurity for aquaculture and is not a scientific study. Based on research (Bowker, et al. 2011), recommends Chlorine 500 mg/L for 15 minutes or Virkon® Aquatic 0.5 to 1% for 10 minutes to disinfect Whirling disease virus, VHS, LMBv, and SVCv.

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<http://www.oie.int/international-standard-setting/aquatic-manual/access-online/>

Direct quotes:

“The virus is inactivated at 56°C for 30 minutes, at pH 12 for 10 minutes and pH 3 for 2 hours

(Ahne, 1986). ”

“The following disinfectants are also effective for inactivation... 540 mg litre–1 chlorine for 20 minutes, 200–250 ppm (parts per million... (Ahne, 1982; Ahne & Held, 1980; Kiryu et al., 2007). ”

“The virus is most stable at lower temperatures, with little loss of titre for when stored for 1 month at –20°C, or for 6 months at –30 or –74°C (Ahne, 1976; Kinkelin & Le Berre, 1974). ”

VHSV reference in the above source was quote from another study Arkush, et. Al 2006, this reference has been added.(75)

30. Iowa State University: College of Veterinary Medicine. 2007. Spring Viremia of Carp.

http://www.cfsph.iastate.edu/Factsheets/pdfs/spring_viremia_of_carp.pdf

Direct Quote:

“It can be inactivated with...chlorine (500 ppm)... SVCV can also be inactivated by heating to 60°C (140°F) for 30 minutes...” No contact time was given for the bleach solution.

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Various chemical and physical methods for destroying the triactinomyxon (TAM) stage of the myxozoan parasite Myxobolus cerebralis were tested at different exposure/doses. Freezing or drying for 1 h, Chlorine concentrations of 130 ppm for 10 min, immersion in 75oC water bath for 5 min all produced 0% viability of parasite which causes whirling disease. However at 58oC water bath for 5minutes, as much as 10% remain possibly viable.

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Direct Quote:

“Immerse gear in a chlorine bleach solution for five minutes (3 cups of household bleach in 5 gallons of

water). Freezing at -4 °F for 24 hours (home freezer) will also kill the spores....completely dry for a minimum of 24 hours for dessication to effectively kill the spores.”

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